



## WELCOME TO ALL ABOUT TITING!

### Note in advance

Since this document also contains images, it is best read on a laptop, PC or tablet. On a PC or laptop you can use the search function here by using Control + F.

This document can already be used, but additions, examples, etc. will be added. Changes will be made automatically to everyone who has shared this document. This means that this document always remains up to date.

### Preface

Welcome to the Facebook group [Everything about Titeren](#). This group was created at the beginning of 2017 for a small group of friends. Not knowing that the need for clear and concise information about titering would be so enormous that there are now more than 20,000 members. This group has given titering a huge boost and we are far ahead of the rest of the world in terms of knowledge. Because it is all volunteer work, we ask everyone to first read this document carefully so that we do not continually receive the same questions. In this document we try to explain to everyone the basics of titering/immune system and the most common questions will be answered here. An important note in advance: titering puppies cannot be compared to titering adult dogs. This is also explained in detail in this document!

### Facebook group and Facebook page!

No information can or may be shared from a Facebook group. That is why we also have a Facebook page in addition to the group. If there is interesting and/or important information that sharing is permitted or even recommended, we will post it on the Facebook page. Of course we hope that you will like not only this but also our [Facebook page Everything about Titeren](#) like!!!! (hint! ;-)

### Search function

Are you looking for something? Then press Control + F (if you are looking on a PC or laptop) and the search bar will appear! Because this document is in Google Docs, it can easily be shared and adjustments will be made everywhere, so this document will always be up to date. So much for the foreword, now on to the titling!

### What is titering?

By taking a few drops of blood from the dog or cat, a titer test can be used to determine whether there are still sufficient antibodies in the blood. We can also check whether an administered vaccination has actually been effective. If the animal is still protected, there is no point in vaccinating again. The vaccination will then add nothing. By titering you can see whether or not vaccination is necessary so that the dog or cat does not have to be stressed unnecessarily and/or unnecessarily. Below we will mainly talk about dogs, but cats can also be assessed!

### Various titer tests

There are 2 different titer tests that veterinarians can use to determine titer in their practice: the VacciCheck and the RapidSTATUS™ TiterTest™. A very small amount is required for both tests



need blood. This will be carried out by the vet and the place where this is done may differ per doctor. It is precisely what this doctor has the most experience with and finds most comfortable. Think of the front leg, back leg, neck, or in the cat's ear.

### Laboratory

Unfortunately, there are still veterinarians who believe that in-house tests such as VacciCheck and RapidSTATUS™ TiterTest™ are not reliable. Both tests are very reliable if performed correctly. There are also laboratories that use these in-house tests! In that case, it will in any case be more expensive because both the vet and the laboratory now perform actions for which you obviously have to pay.

Our experience is that a laboratory result is often taken for information by the veterinarian and is not (correctly) recorded in the animal passport. That makes such a test almost unnecessary because you have paid for it but still do not have a current pet passport and will therefore have to take action again if you have to show the passport somewhere. Conclusion: with one of our veterinarians [TitleList](#) you can simply go and have a titer test done.

### VacciCheck

Until the beginning of 2020, this test was the only titer test that was used in a practice instead of sending blood to a laboratory, which also meant that the costs were/are much higher. For this test, more than 50 actions must be performed very accurately, and the times in particular must be followed very correctly. If this is not done accurately, the result may not be reliable. This test takes approximately half an hour (including drying) and is therefore usually not performed immediately during a consultation. Therefore, practices often collect blood samples from multiple animals to test simultaneously at a later date. This also means that in that case a result will not be given immediately and you will have to return at a later time to collect the completed passport and, if necessary, have it vaccinated. If collection takes place, more blood will also be needed because it must be stored correctly. Of course, there are also veterinarians who immediately use the VacciCheck and you can wait for it, but it is advisable, if you wish, to ask this first when making an appointment for titering. The complexity of this test has led us to set up a list with the aim of offering high quality to our members because too often people were disappointed after discovering that something had gone wrong. We then started with a very extensive knowledge test after which veterinarians could be qualified by name. Because the distributor changed their policy in mid-2019 and went for quantity instead of quality and started using its own list on which everyone who had ever taken a VacciCheck test was placed, regardless of whether they have the necessary knowledge. We were back to square one and from that moment on we decided to stop qualifying veterinarians.

Since March 20, 2021, our group no longer supports the VacciCheck at all for several reasons. These are detailed [in this topic](#) described.

### RapidSTATUS™ TiterTest™

Development does not stand still and this test has been introduced in Europe since the beginning of 2020. This test only requires 3 actions and the result is ready within a few minutes (max. 10 minutes after using the test). Because the test is performed horizontally and the diseases for which the titer is titrated are clearly stated on each cassette, incorrect reading can no longer occur. This test is completely based on the principle of the WSAVA (World Small Animal Veterinary Association), which draws up the Vaccination Guidelines. They consider the presence of antibodies much more important than their level. After all, protected is protected. Compare it with a pregnancy. You are pregnant



or not, but not just a little bit! The RapidSTATUS™ TiterTest™ has been received very enthusiastically in the Netherlands and within a year it has already been used at more than 100 locations.

This test is also extremely suitable to perform during a consultation. Upon arrival, blood can be taken first. Only 3 drops are required for this test. A drop is then placed in each compartment and once it has been absorbed, 2 drops of buffer liquid are added from the bottle. Once these have been completed, the clock can be set to 10 minutes. This means that the vet can now safely examine your dog or carry out the treatment for the reason for the appointment. The test will be ready earlier than the consultation so that the passport can be completed immediately and if vaccination is required, this can be done immediately. If you want to take a photo of the result with the Rapid, this must be done within 20 minutes after the start of the test. The test may then become discoloured, which no longer provides a reliable picture. Partly for this reason, it is not responsible to take a test home, but in addition, the test has been in direct contact with blood, which means that it belongs to risky medical material and must be processed by the veterinarian in a special manner.

### Which antibodies are titered for?

By titrating, the antibodies of the dog of the 3 vaccines contained in the cocktail are measured, namely:

#### **DOG (Canine)**

**Distemper**(Canine Distemper Virus, distemper or Carré disease, abbreviated CDV)

Infectious**Hepatitis**(Canine Adeno-2 Virus or CAV-2; also against CAV-1) **Parvo**(Canine Parvo-2 Virus or CPV-2).

These 3 vaccines are together in the DHP cocktail (first letters of the bold names).

#### **CAT (Feline)**

**19.05.2021:** There are other insights/guidelines for this and we will mention them here as soon as possible.

### Mention in the pet passport

A titer determination should simply be noted in the animal passport so that one has a valid document stating whether or not the animal is optimally protected and what the vet's advice is. Because so many mistakes were/are made with the VacciCheck, we determined in 2017 that we only allow results in the group that are provided with the strip and the values indicated. This way we were able to eliminate a lot of mistakes.

The RapidSTATUS TiterTest cannot be stuck in the booklet and may not be given because there is still blood residue in the sample compartments, so the label that accompanies the test is sufficient. A photo (taken within 20 minutes after the test has been used) of the result is of course always appreciated and is certainly useful to have yourself. Please note that the light can also be distorted in a photo! But a line at the T, no matter how faint, means that antibodies have been detected and the animal is therefore protected.

A titer determination for DHP is generally accepted in almost all (Western) European countries. Always check the LICG website in advance

(<https://www.licg.nl/invoereisen-per-land-europa/>) which requirements are set per country (including countries through which one travels). A valid Rabies vaccination is always mandatory when crossing national borders.



### Can titering also be done for Rabies?

Rabies can be titered (by special laboratories) but is only valid in combination with a valid Rabies vaccination. It is a legal obligation that, as soon as an animal crosses national borders, it must be in possession of a valid Rabies vaccination. Some countries require a positive titer determination in addition to a valid vaccination. Then you can be sure that the vaccination has worked and that the animal in question cannot become ill and pass on the disease. On the LICG website (<https://www.licg.nl/invoereisen-per-land-europa/>) states exactly which requirements are set per country. Also pay close attention when traveling through multiple national borders. Even if you do not get out, the animal must still meet all the requirements of the country in question.

### Why is not Leptospirosis (including Weil's disease) tested?

Because Leptospirosis is a bacterial infection for which no titer determination is possible. This is possible for a viral disease. In addition, the Lepto vaccination is only effective for a shorter period of time (it is a dead vaccine), so the titers also drop relatively faster. Titering therefore makes no sense and would be far too expensive.

In our group we absolutely do not want discussions about Weil/L4, kennel cough or other matters that have nothing to do with titering. Opinions differ enormously on this and it is at the expense of all information about titering and that is often complicated enough. For information: the L4 vaccination is a liquid vaccine and is often used as a solvent for the DHP vaccination. It is therefore not correct that the DHP cannot be given without L4 because it can also be dissolved with a suspending liquid (water for injections).

General: by taking a small amount of blood, it can be measured whether antibodies are found in the blood.

### Titer determination via a laboratory

Veterinarians who do not yet want to titrate themselves send the blood to an external laboratory. Many laboratories (especially in Belgium) now also work with one of the above-mentioned rapid tests. The costs for sending to a laboratory will therefore always be higher because they also have to be paid.

### Differences in reading of both tests

#### VacciCheck

VacciCheck works by assigning values. After comparing with the Gold Standard test, we know that the values are not always consistent, so that, for example, an S3 can also be an S4 or an S2 (when compared with the Gold Standard test). This research did show that positive and negative were generally similar and that is the most important thing. For adult dogs, this means that if all values are S3 or higher, they can be signed off for 3 years. If an S1 or S2 is obtained for one of the diseases, different advice may follow.

#### RapidSTATUS™ TiterTest™

This test works very simply. The C(check) on the test should have 3 red lines so that people know that the test works. Every visible red line at the T (Test line) means that antibodies have been found (even with a very light red line). Completely in accordance with the guidelines of the WSAVA, a positive titer can simply be signed off for 3 years (in an adult animal). Compare it to a pregnancy test: pregnant is pregnant and not just a little bit pregnant!





## Reliability figures

Comparing tests with each other makes no sense. So not one test is 100% and which test would you assume would be the correct one? This is even very risky. Because which test should be the right one? The only way to find out is to have the Gold Standard test performed. This is the 'official' test on which all scientific research is based as a starting point.

Below are the results of these studies.

**RapidSTATUS™ TiterTest™ Canine** beschikt over een hoge **sensitiviteit, specificiteit en nauwkeurigheid**. De sensitiviteit en specificiteit van de test zijn gebaseerd op een 2x2 analyse van gegevens.

Source: CPV/CDV/CAV Antibody Lateral Flow Test Kit - Chinta M. Lamichhane, BVSc & AH, PhD; Adrian Alexandru, DVM, Siba K. Samal, BVSc & AH, PhD, Diplomate ACVM

RapidSTATUS™ TiterTest™ Canine	Sensitiviteit	Specificiteit	Nauwkeurigheid
Canine Distempervirus (CDV)	92%	99%	96%
Canine Parvovirus (CPV)	96%	98%	98%
Canine Adenovirus-2 (CAV-2)	100%	98%	99%

[RapidSTATUS TiterTest sensitivity, specificity and accuracy report](#)

### VacciCheck Canine sensitiviteit en specificiteit (1)

Source: Sensitivity-specificity-accuracy and difference between positive and negative mean results of the ImmunoComb® Canine VacciCheck Antibody Test Kit for Canine Distemper, Parvo and AdenoVirus. (2009) Mazar S.1, Larson L.2 and Lavi Y.3

VacciCheck Canine	Sensitiviteit	Specificiteit	Nauwkeurigheid
Canine Distempervirus (CDV)	88%	100%	onbekend
Canine Parvovirus (CPV)	100%	83%	onbekend
Canine Adenovirus-2 (CAV-2)	96%	82%	onbekend

### VacciCheck Canine sensitiviteit, specificiteit en nauwkeurigheid (2)

Source: A field and experimental trial to assess the performance of the ImmunoComb Canine VacciCheck Antibody Test Kit - Biagal, Galed Labs. Kibbutz Galed, 1924000, Israel

VacciCheck Canine	Sensitiviteit	Specificiteit	Nauwkeurigheid
Canine Distempervirus (CDV)	88%	100%	91%
Canine Parvovirus (CPV)	100%	92%	97%
Canine Adenovirus-2 (CAV-2)	94%	93%	93%

## Is a titer determination officially recognized?

The WSAVA, the KNMvD, the NWWA, the Dibevo and the kennel clubs of the Netherlands (Raad van Beheer), Belgium (KMSH) and Denmark (DKK) have now recognized the titer determination. This means that a valid titer determination is permitted at most events/institutions that fall under these associations.

## Titering boarding houses is permitted

A titer determination is valid at boarding houses and other care facilities for dogs and cats. It goes without saying that the veterinarian must ensure that there is good substantiation and clearly indicate in the animal passport until when this result is valid. If a boarding house does not accept titer determination, it is advisable to ask whether they know whether the vaccinated animals are optimally protected or not. Feel free to point them to our Facebook group because this usually means that they are not yet familiar with the subject. Umbrella trade organization DIBEVO therefore advises its affiliated members to accept a titer determination.



### **Is a titer determination also valid abroad?**

When a dog goes abroad, a valid Rabies vaccination is always an obligation. The titer determination for the DHP is valid in most European countries. Look for the obligations on the various appropriate sites (e.g. [LICG](#) .) and the embassy of the country in question. It is better to be overinformed and well prepared than to encounter problems on the spot with all the consequences that entails.

### **What are the costs for titering?**

These can of course differ per practice. It therefore makes no sense to mention an amount about this. Assume an average amount between 50 and 60 Euro, but in most cases it will not differ much from the amount for a vaccination. However, by titering you know whether the animal is well protected, while you do not know by simply vaccinating. If you are unlucky enough that an animal was not properly protected, there will of course be additional costs for a vaccination and a control titer. But that is exactly the reason why you choose to titer!

### **Is titering reimbursed by pet insurance?**

Yes! All insurers now equate titer determinations to vaccinations for the DHP. Of course, you must have the package that covers vaccinations. If you have any doubts and/or questions, always consult your own insurer!

### **When to start titering? Dogs/cats from 6 months and older**

If your animal has not been titered before, it is advisable to have it done. The largest group of unprotected animals are the young animals that still had sufficient protection from the temporary protection they received from their mother at birth. Please read on to find out exactly how that works! In adult dogs, titering can be done at any time, as long as there is at least 3-4 weeks between the last DHP (or one or more components thereof) and the time of titering.

Preliminary note: both titer tests are ideal for use in puppies.

#### Puppies

More and more breeders are switching to the combination titering/vaccination and having their puppies in the litter tested for the first time at the age of 6-7 weeks. The advantage of this is that there is a very good chance that a puppy that is vaccinated at the right time and the vaccination is immediately successful, will only need 1 vaccination instead of the usual 3.

A puppy receives temporary antibodies from its mother in the colostrum for the first 12 hours. These antibodies from the mother are also called maternal antibodies. If the mother does not have antibodies, she cannot pass them on to her puppies and the puppies are not protected! It is therefore advisable to titrate the expectant mother and vaccinate if necessary before mating takes place. Do this well in advance so that there is still sufficient time to vaccinate and to check whether this vaccination has actually worked. There is no certainty that a well-protected mother will provide her puppies with sufficient maternal antibodies, but what is certain is that what she does not have, she cannot provide. How long the maternal antibodies will be present varies per puppy. Some puppies are already through it at 6 weeks and need to be vaccinated, but there are also puppies that no longer have maternal antibodies until 20 weeks and only then need to be vaccinated. The record in our group is 25 weeks! What is most important to know is that as long as a puppy still has its mother



If you have antibodies, there is a very good chance that a vaccination will not work. That is also one of the reasons that puppies in the Netherlands and Belgium receive a puppy vaccination three times. This *in hope* that one will catch on. Titering has already established that many young dogs walk around (partly) unprotected for the entire first year. You don't want this and it is certainly not the intention of vaccinating 3 times. You don't want to experience parvo and puppies in particular are still very vulnerable because the immune system still has to fully develop, so titering is a good method for this. By titering the body is not unnecessarily burdened and people 'look for' the right vaccination moment. That ideal moment is when the maternal antibodies are completely gone or almost completely gone. This moment is therefore risky but necessary for a vaccination to work. When titering you know that you are in a high-risk period, but by vaccinating indiscriminately you do not know that, while this period does exist (and should exist!). Because Parvo is the most risky, the Parvo titer result is usually taken as a benchmark. Please note: this also depends on the results of the Hepatitis and Distemper, but also on the circumstances in which the puppy is or will be. The next step is determined based on the results. So there will never be a standard advice and/or procedure! Comparing results therefore never makes sense: it is really all tailor-made per puppy!

#### The puppy is 9 weeks old and has already had a vaccination in the litter

If the puppy in the litter has already had the 6 week vaccination (DP or Distemper and Parvo), it is still possible to start titering. In most cases it will make a difference with just 1 vaccination (the 9 week vaccination for Parvo), but that of course depends on the result. The most important thing is and remains to ensure that the puppy is optimally protected and if this is possible with fewer vaccinations, that is only a gain.

Because vaccination has already taken place, in most cases 2 titer determinations will be required before it is known whether any antibodies present come from the 6-week vaccination or whether they are still maternal (temporary antibodies from the mother).

If the titers have decreased during the next titer determination, you can be sure that you are still dealing with maternal antibodies. If the titers are still the same, you can assume that they were due to the previous vaccination. Only with Hepatitis you can be almost certain that it is still maternal because no vaccination has yet been done against it.

#### The puppy is 12 weeks old and has had 2 vaccinations

The puppy has been vaccinated at both 6 and 9 weeks and only needs the 12 week vaccination (which also includes Hepatitis). Is it still useful to titer? Of course! Because no one now knows whether there are still maternal antibodies present that can block the effect of a vaccination. So it is still possible to determine the right time to give that last puppy vaccination based on 1 or 2 titer determinations.

#### The puppy is 12 weeks or older and has had the 3 'puppy' vaccinations

And now comes the key question: 'do you know whether your puppy is now optimally protected?' So no! You hope so but you don't know for sure. That is why it is very wise to have a titer determination done at approximately 16 weeks so that you can be sure that there is optimal protection and that your puppy cannot contract or pass on one of these diseases! The largest group of dogs that are not protected fall into this age category, so don't be surprised if this is the case, but be happy that you started titering because this is exactly why you started titering!

#### 1-year vaccination or titering?

Your dog has had the 3 puppy vaccinations and around/after the age of 1 you will receive a call to be vaccinated again. What is wise: titering or vaccinating? Well, this is always a difficult one because when you have a dog



is well protected, then the vaccination is not necessary at all. But if it turns out that your dog is not well protected, a vaccination is still necessary. We personally recommend titering anyway, for the reasons that fortunately the majority (approx. 80%) is well protected, but also that if 1 or more components are not well protected, it is good to know that and perhaps vaccinations can be perfectly tailored so that not everything is necessary. So that is a decision you have to make yourself!

### Vaccines

Unfortunately, not all vaccines are available as mono-vaccines. The possible combinations of vaccines are:

 <b>RapidSTATUS™ TiterTest™</b>	
NEGATIEF	VACCIN
Parvo	P
Distemper	DP
Hepatitis	DHP
Parvo + Distemper	DP
Distemper + Hepatitis	DHP

**BELANGRIJK:**

3-4 weken na de vaccinatie altijd een controletiter adviseren zodat men zeker weet dat de vaccinatie ook daadwerkelijk is aangeslagen.

### Control titer

A control titer is the titer determination that takes place approximately 3-4 weeks after vaccination. So to check whether this vaccination has worked.

### Marking puppies/young dogs

When the young dog is optimally protected, the vet can sign the pet passport for 1 year. This is because the young animal's immune system still has to fully develop in the first year of life, which means that titers can sometimes drop. Then 1 year later or around 1 year of life is tested again and if the results allow it, you can sign off for 3 years! Don't forget to stop by the bakery because this is worth celebrating!

### Marking adult dogs

When it is certain that the immune system is fully developed, a positive result can be signed off for 3 years. It [WSAVA](#) (Article 86, page 42), this is the worldwide organization that





sets out the guidelines regarding vaccinations, has indicated that in the event of a positive titer result, annual titering is not justified. They also write that the presence of antibodies is more important than their level.

### The Title List!

When we started this group in February 2017, we discovered that a lot went wrong with the implementation and interpretation of VacciCheck. That was the reason why we had ours at the time [TitleList](#) have set up. A list of practices that we knew had the knowledge required to titer. We started with less than 10 practices, but the quality was not improving. We then started by administering a very extensive knowledge test, followed by a telephone evaluation. When they got through that successfully, we placed them on ours [TitleList](#) as a qualified VacciCheck veterinarian. Nevertheless, things still go wrong far too often because, for example, titering is done by another doctor (in the same practice).

We do not mean to say that if someone is not on our list, things never go well. We just want to say that we know from the qualified VacciCheck veterinarians that they understand the whole matter. Our list was also used by the distributor of VacciCheck at the time, but in mid-2019 they decided to make a list themselves. This includes everyone who has ever ordered a VacciCheck, regardless of their knowledge about titering or performing the test. The distributor decided to go for quantity instead of quality. That was the reason for us to stop qualifying. Why should we make an effort for good quality if the supplier does not attach any value to it. We were training the veterinarians and since all the experts have left there, virtually no training is given anymore. And we see that in all the mistakes that are made. Please note: this is not only due to the distributor, it is also due to the relevant doctors who offer the test but have not first delved into the matter.

That is why we were very pleased with the arrival of the new RapidSTATUS™ TiterTest™, which actually immediately eliminates all the disadvantages of the VacciCheck. Due to the simplicity and user-friendliness of this test, no qualification is required and every practice can use it on our website [TitleList](#) come. However, it is absolutely not the intention to get on the list with the RapidSTATUS™ TiterTest™ and then start titering with VacciCheck. Then this practice will be immediately removed from the list. We try to ensure the quality and reliability of our [TitleList](#) to keep it as high as possible. A qualified VacciCheck veterinarian can also disappear from the list if, for example, he or she no longer works in the practice in question or if internal actions go wrong too often. That is why we always recommend that you consult our list again shortly before making an appointment to see if it is still up to date.

Please note: this list is based solely on titering and not on other matters in a practice.

### Legend [TitleList](#)



A green VC logo represents a qualified VacciCheck veterinarian. Make sure you always have an appointment with this qualified veterinarian.



A blue dog paw represents the practices that (also) work with the RapidSTATUS™ TiterTest™.



A purple star represents a titer session by a doctor from our list. A titer session takes place at a location other than a practice. Consider, for example, a pet shop, dog school, etc.

### **VERY IMPORTANT:**

**This list is continuously updated. So always check shortly in advance for any changes are!**

It is not standard for a health examination to take place during titer sessions. This may differ per doctor/organization. Only if vaccination is required, a health check will always take place in advance because unhealthy or sick animals may not be vaccinated.

### **Can titering also be done during a consultation?**

That depends on which titer test is used. If this is done with the RapidSTATUS™ TiterTest™, the doctor will immediately take the blood upon arrival, use the test and perform the physical examination while it is running. The results are ready within 10 minutes. If the result is positive, the passport will be completed immediately and can be taken with you.

With the VacciCheck, blood samples from multiple animals are usually collected to perform the test at a later time. In that case, you will have to return again if you need to be vaccinated and/or to collect your passport. There are also qualified VacciCheck veterinarians who do this during the consultation, so always ask this when making an appointment so that you are not faced with any surprises.

During a titer session, the waiting time is often a little longer (because several animals are titrated at the same time), but the results will then be provided immediately. Usually there is also the option to be vaccinated immediately, if necessary, and the passport is immediately completed and returned.

A word of advice: consider what the body has to process during a vaccination. That is why we are in favor of not giving multiple vaccines at the same time. The vaccinations for Lepto and Rabies in particular are very stressful. It is therefore advisable to administer this several weeks apart, separately from other vaccinations.

### **Distributors**

The websites of the distributors themselves also contain a lot of information about the tests:

RapidSTATUS™ TiterTest™: [www.titertests.nl](http://www.titertests.nl)

Vaccicheck: [www.vaccicheck.nl](http://www.vaccicheck.nl) (note: this shows a list of all practices that have ever administered a test, but this says nothing about the quality of the knowledge and/or implementation. This also lists all practices where we see many mistakes).

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